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Alterations of host resistance to *Listeria monocytogenes* in tumour-bearing mice and the effect of *Corynebacterium parvum*

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Summary. In tumour (fibrosarcoma)-inoculated mice, alterations of host resistance to *Listeria monocytogenes* following tumour growth were examined. Non-immune macrophage-mediated antibacterial resistance was severely suppressed up to day 4 or so after tumour inoculation, but was enhanced thereafter. On the other hand, T cell-mediated immune resistance retained the control level up to day 7 or so, but was suppressed thereafter. Suppression of macrophage-mediated antibacterial resistance was not observed if the tumour-bearing mice had been pretreated with *Corynebacterium parvum*. Moreover, this suppression of macrophage-mediated resistance was attributable to the presence of a serum factor that interferes with the function of free macrophages but not with that of resident macrophages. The ability of this serum factor to suppress macrophage-mediated antibacterial resistance, however, was not reduced by *C. parvum* administration.

INTRODUCTION

Tumour-bearing animals have been known to develop a depressed ability to mobilize macrophages to inflammatory sites *in vivo* (Bernstein, Zbar & Rapp, 1972; Normann & Sorkin, 1976; Snyderman, Pike, Blaylock & Weinstein, 1976) and have defective chemotactic responsiveness of their macrophages *in vitro* (Stevenson & Meltzer, 1976; Meltzer & Stevenson, 1977). Furthermore, factors that inhibit these functions of macrophages have been recognized in the supernatants of sonicated neoplastic cells (Pike & Snyderman, 1976; Snyderman & Pike, 1976) and in the culture supernatants of rapidly proliferating cell lines (Normann & Sorkin, 1977). Suppression of these macrophage functions in tumour-bearing mice seems to be strictly related to the immediate outcome of depressed macrophage-mediated antibacterial resistance after subcutaneous (s.c.) inoculation of tumour cells reported by North, Kirstein & Tuttle (1976a). They reported also that this state of negative resistance was soon replaced by a contrasting state of enhanced antibacterial resistance (North, Kirstein & Tuttle, 1976b).

Mitsuyama, Takeya, Nomoto & Shimotori (1978) have reported previously that in mice, resistance to *Listeria monocytogenes* was mediated by non-immune resident and free macrophages up to day 3 of infection, and by immune macrophages activated by sensitized T lymphocytes thereafter. Therefore, the effect of a tumour-bearing state on the antibacterial resistance has to be observed not only at an early but also late phase of infection.

In the present study, we examined the time course of the bacterial growth in the liver of mice bearing

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syngeneic tumours at various stages. To investigate the effect of the tumour-bearing state on resistance to bacteria in mice whose macrophages were in a non-specifically activated state, we also examined the time course of the bacterial growth in the liver of tumour-bearing mice injected with *Corynebacterium parvum* before the inoculation of the tumour. In addition, mechanisms of the suppression of macrophage-mediated antibacterial resistance in tumour-bearing mice and of the protective effects by *C. parvum* against this suppression were examined.

MATERIALS AND METHODS

Mice

Male mice of an inbred BALB/c strain were purchased from Shizuoka Union for Experimental Animals, Japan. They were employed in experiments at 8–10 weeks of age. Each experimental group consisted of six mice.

Tumour

The 3-methylcholanthrene-induced Meth A fibrosarcoma, syngeneic to BALB/c mice, was donated from Aichi Cancer Center Research Institute, Japan. The tumour was maintained in an ascites form by weekly passage. In all experiments, 2×10^6 tumour cells were inoculated s.c.

Bacteria

L. monocytogenes (EGD), prepared as previously described (Mitsuyama *et al.*, 1978), was used for experiments. The LD50 after intravenous (i.v.) injection was approximately 2×10^4 viable bacteria in normal male BALB/c mice.

Corynebacterium parvum

A suspension of heat- and formalin-killed *C. parvum* (IM 1585) containing 2 mg/ml dry weight of organisms was supplied commerically by Institute Merieux, Lyon, France. Mice were usually injected i.v. with 0·5 ml (1·0 mg) of this suspension 7 days before the inoculation of tumour cells. Enhanced resistance to *L. monocytogenes* was observed from about day 4 to 21 after injection with *C. parvum* in normal mice.

Measurement of bacterial growth

Mice were injected i.v. with appropriate sublethal or lethal doses of viable bacteria in phosphate-buffered saline (PBS). At various times after injection, determination of bacterial growth in the liver was performed as previously described (Mitsuyama *et al.*, 1978).

Serum transfer

Tumour-inoculated donor mice pretreated with *C. parvum* or not were bled to obtain serum 24 hr after tumour inoculation. Serum was also obtained from normal mice or from those pretreated with *C. parvum* alone as controls. Syngeneic normal recipients were injected intraperitoneally (i.p.) with 0·1 or 0·5 ml of these sera and 1 hr later, injected i.v. with 2×10^3 bacteria.

Statistics

Values were expressed as means ± standard deviations, and the significance of differences between experimental and control groups was analysed using the Student's *t* test. A *P* value of less than 0·05 was taken as significant.

RESULTS

Alterations of host resistance to *L. monocytogenes* at various stages after tumour inoculation

Mice were injected with 2×10^3 viable bacteria at five different times after tumour inoculation and bacterial growth in their livers was examined 1, 3, 7 and 10 days after the infection. When mice were injected with bacteria 5 hr after tumour inoculation, the number of bacteria in tumour-bearing mice strikingly increased up to day 3 of infection in contrast to normal mice, in which the number of bacteria increased slightly, but thereafter began to decrease to reach almost the level of normal mice by day 7 (Fig. 1a). The number of bacteria continued to decrease from day 7 to day 10 in normal mice, but increased slightly from day 7 to day 10 in tumour-bearing mice. In tumour-bearing mice pretreated with *C. parvum*, the number of bacteria scarcely increased from the beginning of infection just as observed in normal mice pretreated with *C. parvum* alone.

When mice were injected with bacteria 3 days after tumour inoculation, the number of bacteria in tumour-bearing mice was larger than that of normal mice on day 1 of infection (4 days after tumour inoculation), but became smaller on day 3 (6 days after tumour inoculation, Fig. 1b). The number of bacteria in tumour-bearing mice, however, did not decrease by day 7 in contrast to normal mice in which the number

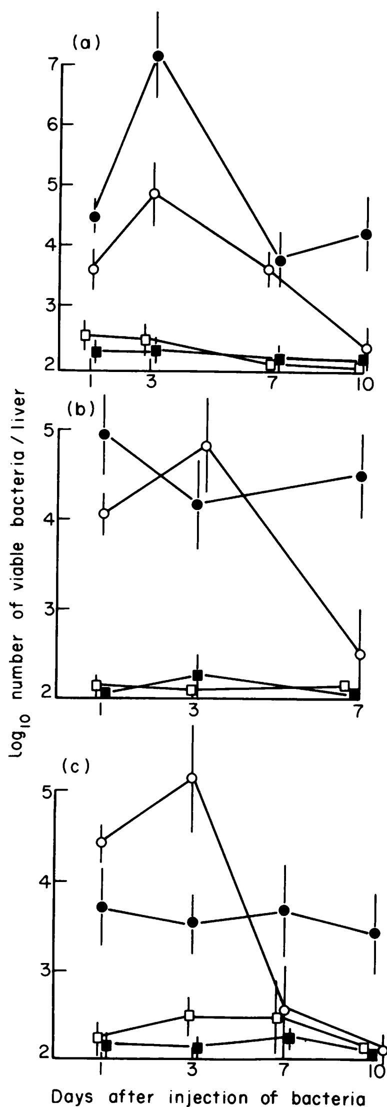

Figure 1. Bacterial growth in the liver of tumour-bearing mice and effect of *C. parvum* on it. BALB/c normal of *C. parvum*-pretreated mice were inoculated s.c. with 2×10^6 Meth A cells (a) 5 hr before, (b) on day −3, (c) on day −7, and they were injected i.v. with 2×10^3 viable bacteria on day 0. (○) Normal mice; (●) tumour-bearing mice; (□) mice pretreated i.v. with 1·0 mg of *C. parvum* on day (a) −7, (b) −10, (c) −14; (■) tumour-bearing mice pretreated i.v. with 1·0 mg of *C. parvum* on day (a) −7, (b) −10, (c) −14. Each point and vertical bar represents the mean result for six mice ± standard deviation.

of bacteria decreased rapidly from day 3 to day 7. The number of bacteria in groups pretreated with *C. parvum* scarcely increased from the beginning of infection irrespective of tumour inoculation.

When mice were injected with bacteria 7 days after tumour inoculation, the number of bacteria in tumour-bearing mice was significantly smaller than that in normal mice during the first 3 days of infection, but thereafter did not decrease in contrast to that in normal mice (Fig. 1c). The number of bacteria in groups pretreated with *C. parvum* scarcely increased from the beginning of infection irrespective of tumour inoculation as shown in Fig. 1a and b. When mice were injected with bacteria 14 or 21 days after tumour inoculation, the time courses of bacterial growth in tumour-bearing mice pretreated with *C. parvum* or not were similar to those shown in Fig. 1c (data are not shown).

The capacity of resident macrophages to kill bacteria in mice bearing tumours at various stages

As reported previously, Mitsuyama *et al.* (1978) in our laboratory showed that approximately 90% of viable cells of *L. monocytogenes* were trapped in the liver within 10 min after i.v. injection and about 90% of them were killed by resident macrophages of the liver within 6 hr. Consequently, an experiment was conducted to find out whether or not the modification of bacterial growth during the first 3 days of infection in tumour-bearing mice as shown in Fig. 1a, b and c was due to the changes in the function of these resident macrophages. Mice were inoculated with tumour cells on day −1, −3, −7, −14 or −21 and injected with 3×10^5 bacteria on day 0. The number of bacteria in the liver was examined 6 hr after bacterial infection. The numbers of bacteria in these groups of tumour-bearing mice were almost the same as that in normal mice (Table 1). When mice were pretreated with *C. parvum* on day −8, the number of bacteria was smaller than that in non-treated normal mice. Tumour inoculation on day −1 did not affect the enhanced antibacterial resistance in *C. parvum*-pretreated mice. Thus, the modification of bacterial growth during the first 3 days of infection in tumour-bearing mice may not be ascribed to the modified function of resident macrophages. On the other hand, the enhanced antibacterial resistance in *C. parvum*-pretreated mice may depend at least partly on the activation of resident macrophages in the liver.

Table 1. Constant killing of bacterial inoculum by resident macrophages in tumour-bearing mice and the effect of *C. parvum*

Pretreatment with *C. parvum*	Day of tumour inoculation	Log_{10} number of viable bacteria/liver
−	Not inoculated	4·40±0·20
+*	Not inoculated	3·64±0·24†
−	−1	4·33±0·11
+*	−1	3·66±0·12†
−	−3	4·43±0·14
−	−7	4·53±0·120
−	−14	4·50±0·18
−	−21	4·34±0·14

Mice were injected i.v. with 3×10^5 bacteria on day 0, and bacterial counts were performed 6 hr after.

* Mice were pretreated with *C. parvum* on day −8.

† Both significantly lower than that of normal mice ($P < 0{\cdot}05$, two-tailed t test).

Effect of *C. parvum* on the ability of serum factor in tumour-bearing mice to suppress antibacterial resistance

A circulating serum factor which suppresses macrophage-mediated antibacterial resistance has been recognized in tumour-bearing mice (North *et al*, 1976a). Therefore, the ability of serum of tumour-bearing mice pretreated with *C. parvum* to suppress macrophage-mediated antibacterial resistance was investigated. As shown in Table 2, bacterial growth during the first 3 days of infection was enhanced to almost the same extent in recipients transferred with serum from non-treated tumour-bearing mice and in those transferred with serum from *C. parvum*-pretreated tumour-bearing mice. In addition, serum of normal mice pretreated with *C. parvum* alone exhibited no enhancing effect on bacterial growth in recipients.

DISCUSSION

In Meth A-bearing BALB/c mice, macrophage-

Table 2. Effect of *C. parvum* on the ability of serum from tumour-bearing mice to suppress antibacterial resistance

	Log_{10} number of viable bacteria/liver of recipients§	
	Volumes of serum transferred	
Serum donors	0·1 ml	0·5 ml
Normal mice	4·34+0·43	4·35+0·28
Pretreated with *C. parvum* alone*	4·36+0·14	4·54+0·46
Tumour-bearers†	5·88+0·13¶	7·48+0·61**
Tumour bearers pretreated with *C. parvum*‡	6·02+0·48¶	7·02+0·42**

* Mice pretreated i.v. with *C. parvum* 8 days before the harvesting of serum.

† Mice inoculated s.c. with 2×10^6 Meth A cells 1 day before the harvesting of serum.

‡ Mice pretreated and inoculated 8 days and 1 day before the harvesting of serum, respectively.

§ Syngeneic normal mice were transferred i.p. with 0·1 or 0·5 ml of serum and 1 hr later they were injected i.v. with 2×10^3 bacteria. Bacterial counts were performed after 3 days.

¶ and ** not significantly different ($P > 0{\cdot}05$).

mediated antibacterial resistance up to day 3 of infection and T cell-mediated immune resistance as demonstrated by the elimination of bacteria at 5, 7 or 10 days of infection (Mitsuyama *et al.* 1978) were each modified following tumour growth as follows. Macrophage-mediated resistance is markedly depressed from a very early stage to 3 or 4 days after tumour inoculation (Fig. 1a and b), but enhanced at 6 or 7 days and retains the enhanced level thereafter (Fig. 1b and c). T cell-mediated resistance continues to be suppressed from a relatively early stage to a late stage of tumour-bearing state. Tumour-bearing mice at late stages showed a persistent infection-like pattern.

Suppression of macrophage-mediated antibacterial resistance observed at an early stage after tumour inoculation seems not to be due to the depressed function of resident macrophages in the liver, since the bacterial killing depending on resident macrophages as observed during the first 6 hr of infection was not suppressed in tumour-bearing mice (Table 1). Snyderman *et al.* (1976) reported that implantation of neoplasms in mice induced a rapid and dramatic depression in the ability to mobilize macrophages to the sites of delayed inflammatory reactions *in vivo*. There have been many reports indicating the depression in the migratory activity and chemotactic responsiveness of macrophages in tumour-bearing mice, some of which are mentioned in the introduction. Taking the conclusions of those papers into consideration, the suppression of macrophage-mediated antibacterial resistance presented in this paper can be interpreted as being due to the depression of macrophage accumulation to infective foci in the liver.

In tumour-bearing mice pretreated wtih *C. parvum*, the suppression of macrophage-mediated antibacterial resistance was not observed (Fig. 1a, b and c). There was a possibility that this blocking of the suppressive effect of *C. parvum* could be ascribed to reduction in a serum factor capable of suppressing macrophage-mediated resistance. This possibility, however, can be excluded, since the serum from *C. parvum*-pretreated tumour-bearing mice exhibited the same level of suppressive effect as the serum from non-treated tumour-bearing mice in a serum transfer experiment (Table 2). We reported previously that enhancement of resistance to *L. monocytogenes* by *C. parvum* in normal mice was due mainly to the direct non-specific activation of both fixed and free macrophages (Miyata, Nomoto & Takeya, 1980). Furthermore, non-specific activation with *C. parvum* has been known to be accompanied with an increase in chemotactic responsiveness of macrophages (Sher, Poplack Blaese, Brown & Chaparas, 1977). Therefore, it seems likely that macrophages activated non-specifically by *C. parvum* could overcome the suppressive effect of the serum factor and exert fully their function to eliminate bacteria even in the presence of the serum factor.

As shown in Fig. 1b and c, a suppressed state of macrophage-mediated antibacterial resistance was converted to an enhanced state from day 6 or so after tumour inoculation. North *et al.* (1976b) also reported this conversion from a suppressed to an enhanced state in tumour-bearing mice and suggested that enhanced resistance was ascribable to the activation of macrophages involved in concomitant immunity to the tumour itself. In the present study, the function of resident macrophages in mice bearing tumours at 7, 14 or 21 days after inoculation was still at the level of that seen in non-treated normal mice, though their macrophage-mediated resistance was in an enhanced state (Table 1). Thus, it seems likely that the enhanced macrophage-mediated resistance observed from 6 days after tumour inoculation can be ascribed to an enhanced ability of free macrophages to accumulate at the site of infection. Stevenson & Meltzer (1976), however, reported that depressed chemotactic response of macrophages in tumour-bearing mice persisted until the death of the hosts. The basis of this discrepancy is unknown at present.

T cell-mediated immune resistance to *L. monocytogenes* began to be depressed from a relatively early stage after tumour inoculation. By this time, however, macrophage-mediated resistance had already been converted to an enhanced state, which continued thereafter.

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REFERENCES

Bernstein I.D., Zbar B. & Rapp H.J. (1972) Impaired inflammatory response in tumour-bearing guinea pigs. *J. natn. Cancer Inst.* **49**, 1641.

Meltzer M.S. & Stevenson M.M. (1977) Macrophage function in tumour-bearing mice: tumoricidal and chemotactic responses of macrophages activated by infection with *Mycobacterium bovis*, strain BCG. *J. Immunol.* **118**, 2176.

MITSUYAMA M., TAKEYA K., NOMOTO K. & SHIMOTORI S. (1978) Three phases of phagocyte contribution to resistance against *Listeria monocytogenes*. *J. Gen. Microbiol.* **106**, 165.

MIYATA H., NOMOTO K. & TAKEYA K. (1980) Characteristics of resistance to *Listeria monocytogenes* enhanced by *Corynebacterium parvum* in mice. *Immunology*, **40**, 33.

NORMANN S.J. & SORKIN E. (1976) Cell-specific defect in monocyte function during tumour growth. *J. natn. Cancer Inst.* **57**, 135.

NORMANN S.J. & SORKIN E. (1977) Inhibition of macrophage chemotaxis by neoplastic and other rapidly proliferating cells in vitro. *Cancer Res.* **37**, 705.

NORTH R.J., KIRSTEIN D.P. & TUTTLE R.L. (1976a) Subversion of host defense mechanisms by murine tumours. I. A circulating factor that suppresses macrophage-mediated resistance to infection. *J. exp. Med.* **143**, 559.

NORTH R.J., KIRSTEIN D.P. & TUTTLE R.L. (1976b) Subversion of host defense mechanisms by murine tumours. II. Counterinfluence of concomitant antitumor immunity. *J. exp. Med.* **143**, 574.

PIKE M.C. & SNYDERMAN R. (1976) Depression of macrophage function by a factor produced by neoplams: a mechanism for abrogation of immune surveillance. *J. Immunol.* **117**, 1243.

SHER N.A., POPLACK D.G., BLAESE R.M., BROWN T.M. & CHAPARAS S.D. (1977) Effect of *Corynebacterium parvum*, methanol-extraction residue of BCG and Levamisole on macrophage random migration, chemotaxis and pinocytosis. *J. natn. Cancer Inst.* **58**, 1753.

SNYDERMAN R. & PIKE M.C. (1976) An inhibitor of macrophage chemotaxis produced by neoplasms. *Science*, **192**, 370.

SNYDERMAN R., PIKE M.C., BLAYLOCK B.L. & WEINSTEIN P. (1976) Effects of neoplasms on inflammation: depression of macrophage accumulation after tumour implantation. *J. Immunol.* **116**, 586.

STEVENSON M.M. & MELTZER M.S. (1976) Depressed chemotactic responses *in vitro* of peritoneal macrophages from tumour-bearing mice. *J. natn. Cancer Inst.* **57**, 847.